Supplemental Information

Suppression of MEHMO Syndrome mutation in eIF2 by small molecule ISRIB

Sara K. Young-Baird, Maíra Bertolessi Lourenço, Megan K. Elder, Eric Klann, Stefan Liebau, and Thomas E. Dever

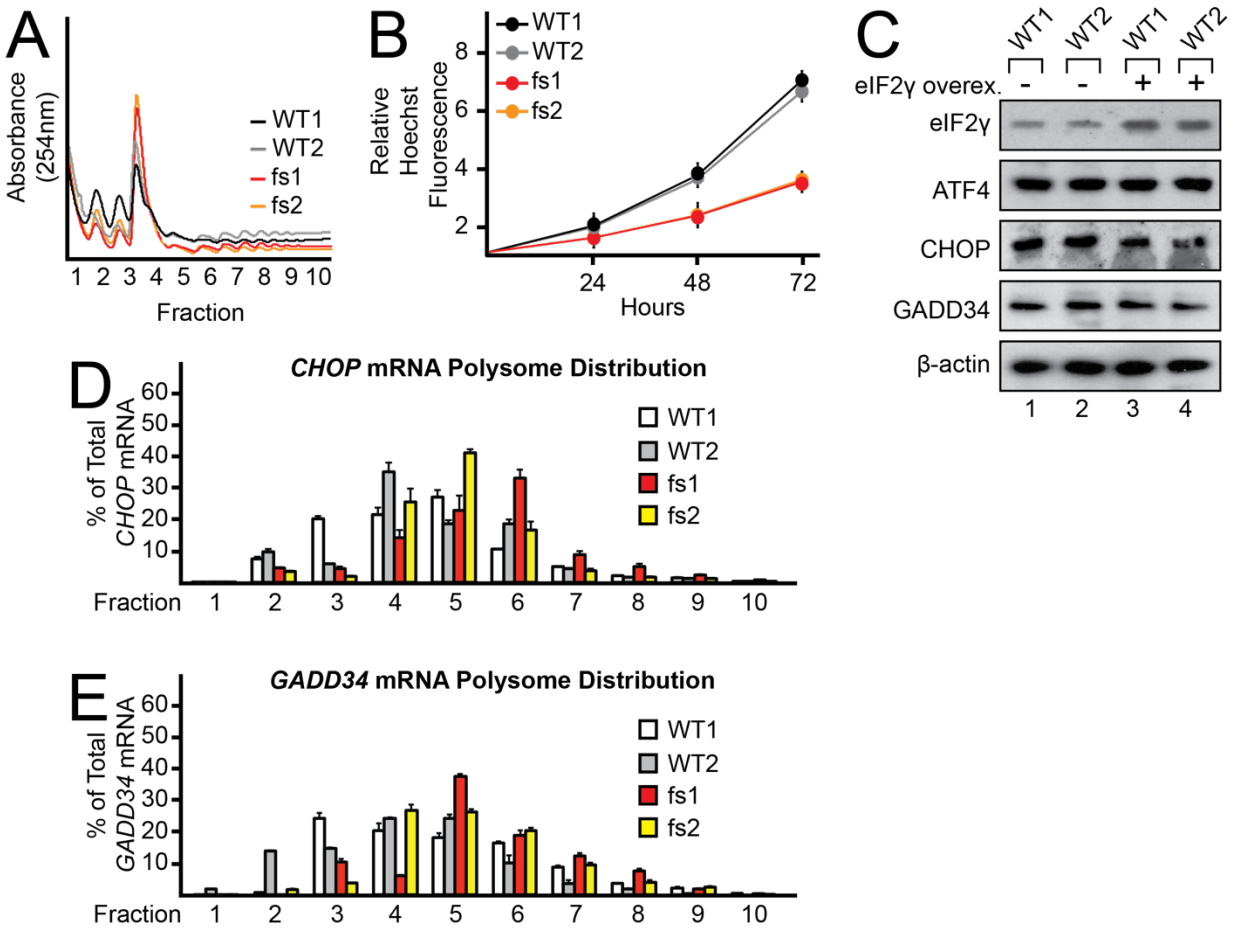


Figure S1. Cell proliferation and translation of *ATF4*, *CHOP*, and *GADD34* mRNAs is enhanced in eIF2γ-fs iPSCs – Related to Figure 1.

(A) Overlay of polysome profiles from Figure 1A.

(B) Equal numbers of eIF2γ and eIF2γ-fs iPSCs were seeded in 96-well plates and allowed to settle for 6 h before time 0 measurements of Hoechst fluorescence were conducted. Cells were grown for 24, 48, or 72 h, with measurement of Hoechst fluorescence at each timepoint. Average values are represented relative to the time 0 measurement for each clonal line. Error bars represent standard deviation (n = 3).

(C) Immunoblot analysis for eIF2γ, ATF4, CHOP, GADD34, and β-actin in lysates from eIF2γ iPSCs overexpressing eIF2γ. Experiment was performed two times with comparable results.

(D-E) Percentage of total *CHOP* (C) or *GADD34* (D) mRNAs in each fraction of a sucrose gradient was determined by qRT-PCR. Error bars represent standard deviation (n = 3). For *CHOP,* 47.8±1.2% of the mRNA was polysome associated in WT iPSCs versus 71.8±5.9% in eIF2γ-fs iPSCs. For *GADD34,* 47.2±7.0% of the mRNA was polysome associated in eIF2γ iPSCs versus 74.5±11.5% in eIF2γ-fs iPSCs.

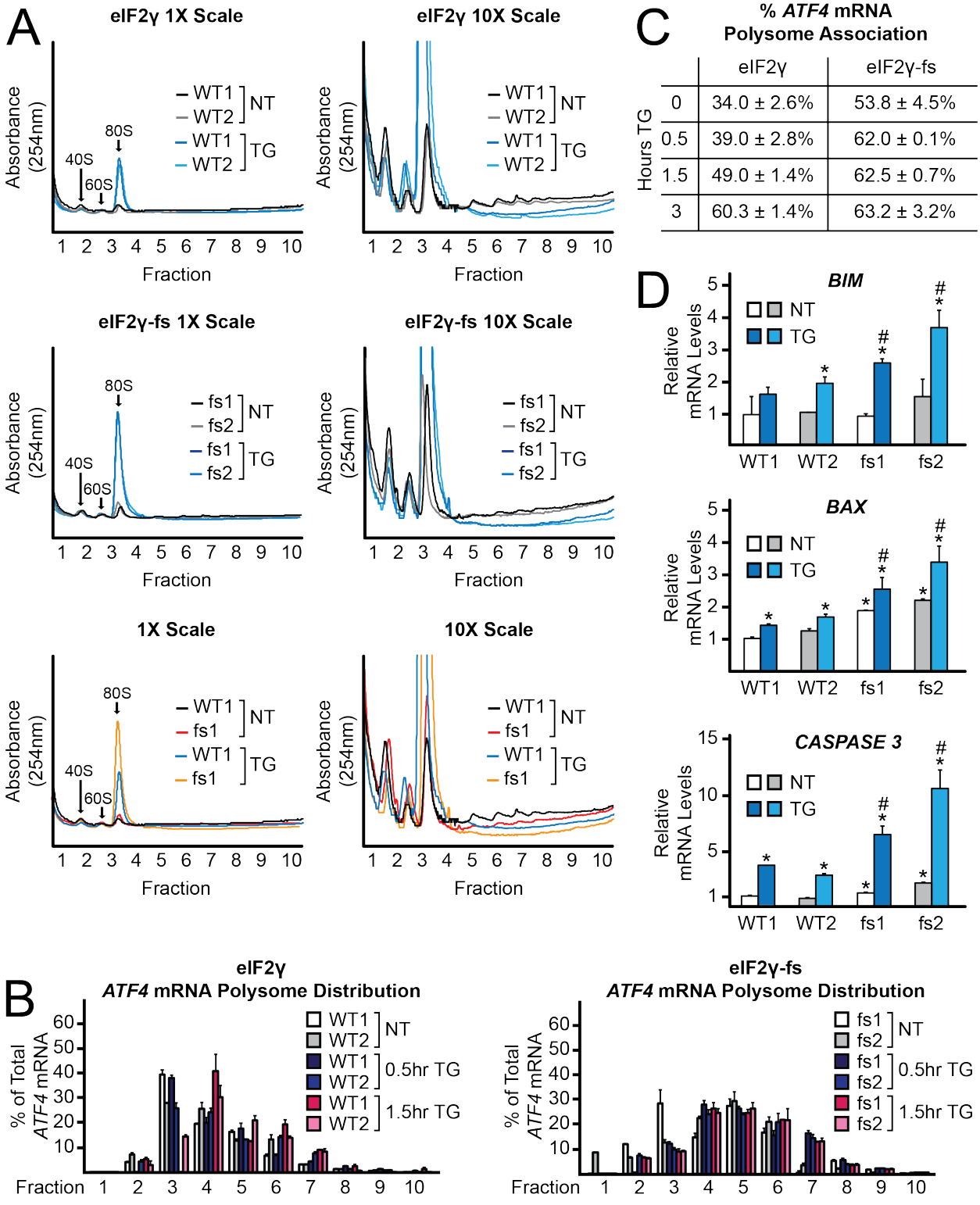
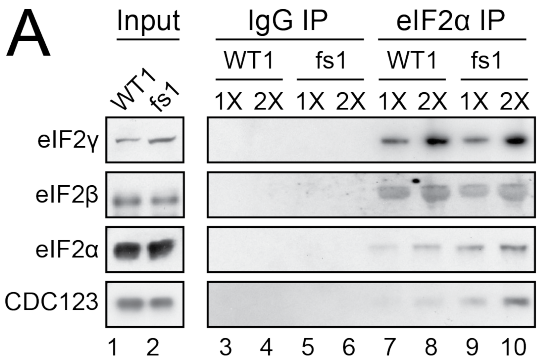


Figure S2. eIF2γ-fs mutation modifies the dynamics of the ISR, promoting chronically low levels of translation and heightened translation of *ATF4* in response to eIF2α–P – Related to Figure 3.

(A) Polysome analysis of lysates from eIF2γ and eIF2γ-fs iPSCs, either left untreated or treated with TG for 3 h. Lysates were layered on 10-50% sucrose gradients and subjected to ultracentrifugation followed by fractionation while monitoring OD at 254nm. Polysome profiles are depicted on either a 1X scale, or a magnified 10X scale to assess changes in the polysome fractions. Peaks for 40S and 60S ribosomal subunits, and the 80S monosome are indicated on the 1X scale profiles. Average polysome/monosome (P/M) ratios with standard deviations (n = 3) are as follows: eIF2γ NT, 2.51±0.09; eIF2γ TG, 0.66±0.02; eIF2γ-fs NT, 1.46±0.26; eIF2γ-fs TG, 0.29±0.16.
(B) Lysates from eIF2γ and eIF2γ-fs iPSCs that were left untreated, treated with TG for 0.5 h, or treated with TG for 1.5 h were fractionated on sucrose gradients. Total RNA was isolated from each fraction, and the percentage of total *ATF4* mRNA in each fraction was determined by qRT-PCR; error bars represent standard deviation (n = 3).
(C) Quantitation of the percentage with standard deviation of *ATF4* mRNA associated with polysomes for eIF2γ and eIF2γ-fs iPSCs (from Panel B) that were left untreated or treated with TG for 0.5, 1.5, or 3 h, as indicated.
(D) Total RNA was collected from eIF2γ and eIF2γ-fs iPSCs either left untreated or treated with thapsigargin for 3 h. Levels of *BIM*, *BAX*, and *CASPASE 3* mRNAs were determined by qRT-PCR and normalized relative to *ACTB* mRNA. *values statistically different from WT1 NT; # fs1 and fs2 TG values statistically different from fs1 and fs2 NT, $p<0.05$ (n = 3).

A



B

Immunoblot Quantification for Figures 4B and S3A

Figure	Comparison	WT1	fs1
4B	eIF2α / eIF2β	1.10 ± 0.14	0.59 ± 0.05
4B	CDC123 / eIF2β	1.02 ± 0.03	2.44 ± 0.04
S3A	CDC123 / eIF2α	0.93 ± 0.06	1.25 ± 0.19
S3A	eIF2γ / eIF2α	1.00 ± 0.08	0.36 ± 0.05
S3A	eIF2β / eIF2α	1.02 ± 0.02	0.42 ± 0.03

C

Immunoblot Quantification for Figure 4C

Comparison	eIF2α overex.	WT1	fs1
eIF2α / eIF2β	-	1.04 ± 0.01	0.56 ± 0.03
eIF2α / eIF2β	+	0.94 ± 0.07	0.85 ± 0.07
CDC123 / eIF2β	-	1.00 ± 0.01	1.97 ± 0.04
CDC123 / eIF2β	+	0.88 ± 0.06	1.12 ± 0.23
Met-tRNA$_i^{Met}$ / eIF2β	-	1.01 ± 0.01	0.18 ± 0.07
Met-tRNA$_i^{Met}$ / eIF2β	+	0.92 ± 0.02	0.53 ± 0.04

D

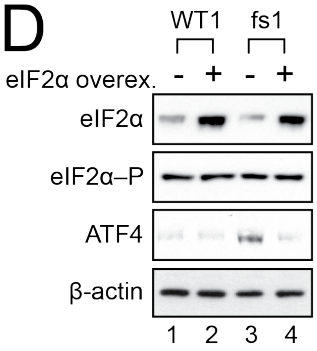


Figure S3. eIF2α overexpression suppresses expression of *ATF4*, *CHOP*, and *GADD34* in eIF2γ-fs iPSCs – Related to Figure 4.

(A) Lysates from eIF2γ and eIF2γ-fs iPSCs were subjected to co-immunoprecipitation with eIF2α or control IgG antibodies. Co-immunoprecipitation samples were divided and either 1X or 2X volumes were subjected to immunoblot analyses to detect eIF2 subunits and chaperone CDC123 in input and immunoprecipitated (IP) samples, as indicated.

(B-C) Quantification of immunoblots from Figures 4B (B), 4C (C), and S3A (B). Values shown are from at least three replicates and are relative to co-immunoprecipitated eIF2β or eIF2α, as indicated.

(D) Lysates from eIF2γ and eIF2γ-fs iPSCs overexpressing eIF2α were subjected to immunoblot analysis for the indicated proteins.

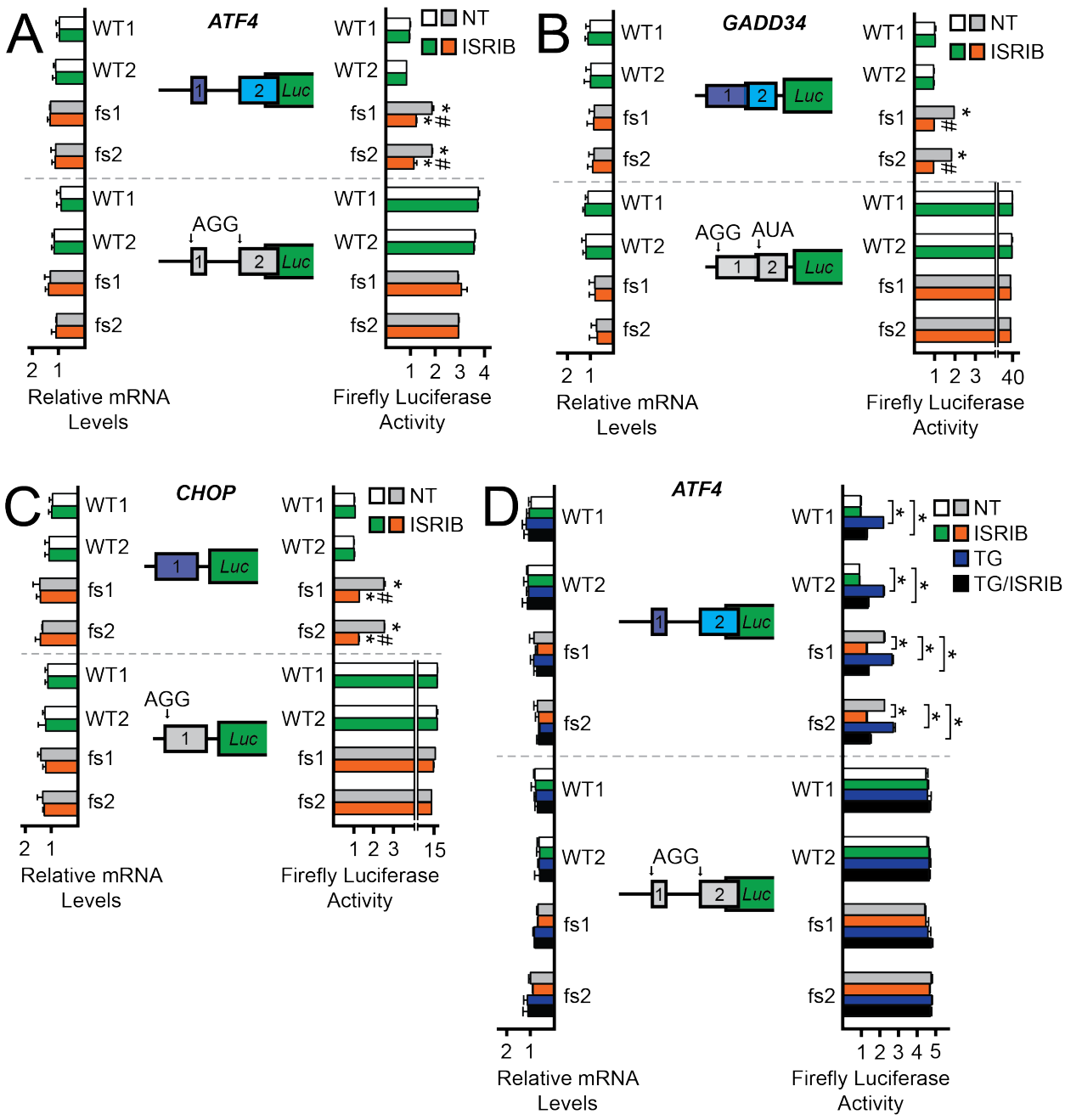


Figure S4. ISRIB reduces uORF-mediated translation of *ATF4*, *GADD34* and *CHOP* mRNAs in eIF2γ-fs iPSCs – Related to Figure 5.

WT and mutant versions of P_{TK}-ATF4-Luc **(A and D)**, P_{TK}-GADD34-Luc **(B)**, and P_{TK}-CHOP-Luc **(C)** were transfected into eIF2γ and eIF2γ-fs iPSCs. Cells were left untreated or treated for either 12 hours (A-C) or 6 hours (D) with ISRIB, TG, or ISRIB plus TG, and then reporter expression and mRNA levels were measured via Firefly luciferase assay and qRT-PCR. The mutant versions of the reporters were described in Figure 2. Average values are represented relative to WT1 containing the respective WT 5'-leader construct; error bars represent standard deviation (n = 3); *values statistically different from WT1; # fs1 and fs2 TG values statistically different from fs1 and fs2 NT, $p<0.05$.

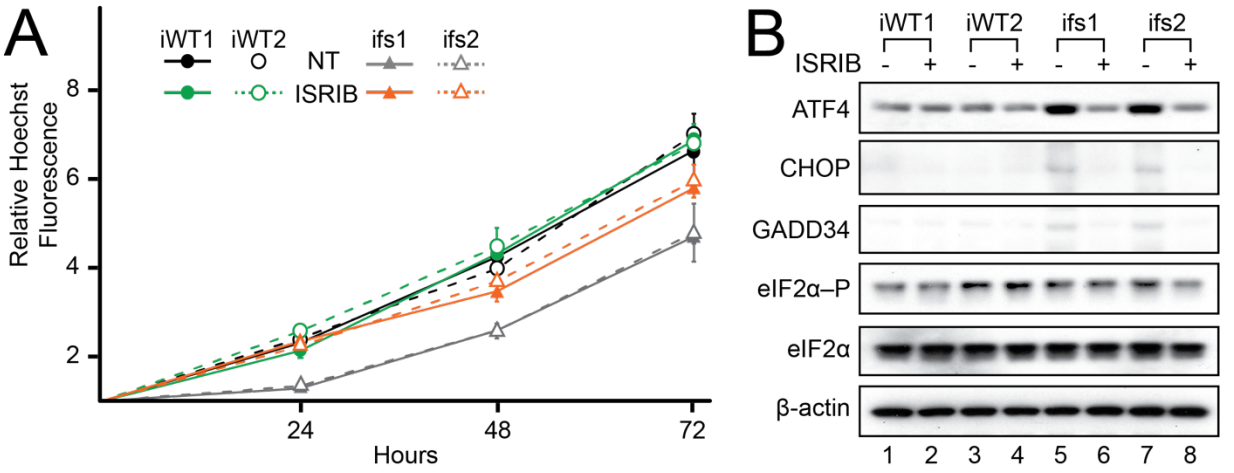


Figure S5. ISRIB restores cell proliferation and ISR signaling to WT levels in isogenic eIF2γ-fs iPSCs – Related to Figure 6.

(A) Isogenic eIF2γ (iWT1 and iWT2) and eIF2γ-fs (ifs1 and ifs2) cell lines were derived from the mutant fs1 cell line. Equal numbers of eIF2γ and eIF2γ-fs iPSCs were seeded in 96-well plates and allowed to settle for 6 h before time 0 measurements of Hoechst fluorescence were conducted. Cells were grown for an additional 24, 48, or 72 h with or without ISRIB treatment, and Hoechst fluorescence was measured at each timepoint. Average values are represented relative to the time 0 measurement for each clonal line. Error bars represent standard deviation (n = 3).

(B) Lysates from isogenic eIF2γ and eIF2γ-fs iPSCs left untreated or treated with ISRIB for 12 h were subjected to immunoblot analysis for the indicated proteins.